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**Oviposition preference of *Anthocoris nemoralis* and *A. nemorum* (Heteroptera: Anthocoridae) on pear leaves depending on leaf damage, honeydew and prey eggs**

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## Summary

Anthocorids are important predators of insect pests in pome fruit. Females insert their eggs in leaf tissue. Their choice of oviposition site determines the later distribution of nymphs. In two-choice experiments comparing oviposition on treated and healthy pear leaves it was tested whether a) the oviposition preference of *Anthocoris nemoralis* and *A. nemorum* was affected by simulated insect damage and whether the oviposition preference of *A. nemoralis* was affected by b) honeydew and c) prey, *Cacopsylla pyri* eggs. Honeydew was applied along the ventral midvein. Prey eggs were placed either along the ventral or the dorsal midvein. While *A. nemoralis* preferred damaged leaves; this was not the case for *A. nemorum*. Honeydew treated leaves attracted more oviposition. On honeydew-treated leaves significantly more eggs were laid on the ventral than on the dorsal leaf blade. Prey infestation attracted more oviposition when prey was placed along the ventral midvein. On infested leaves females laid more eggs along the ventral than the dorsal midvein. With prey along the dorsal midvein, no preference was found, but on infested leaves more eggs were laid along the dorsal rather than the ventral midvein. Results show that prey cues and presence of prey guide predator oviposition, even within the single leaf. The perspectives for biological control in orchards are discussed.

## Introduction

Two of the most abundant predators in apple and pear orchards of Europe are *Anthocoris nemorum* (L.) and *A. nemoralis* (Fabricius) (Anthocoridae: Heteroptera) (Skanland, 1981; Solomon, 1982). Both have a wide range of prey and habitats and oviposition occurs on various plants (Anderson, 1962a; Collyer, 1967; Scutareanu *et al.*, 1994). *Anthocoris nemoralis* is mostly found on perennials, *A. nemorum* is found on both perennials and annuals (Anderson, 1962a).

Several studies have documented the distribution of anthocorids among various habitats and annual changes in distribution among habitats (Anderson, 1962a; Collyer, 1967; Fauvel, 1999). An understanding of the mechanisms that guide the distribution of anthocorids may be an important tool to improve biological control in orchards. One mechanism that can greatly influence the distribution of anthocorids is the female anthocorids choice of oviposition site, which determines resulting densities of the less mobile, but much more numerous, offspring on a given plant or in a given habitat.

A predator's choice of oviposition site may be affected by various factors. Plant species is one such factor. Thus, in a choice experiment *A. nemorum* preferred to oviposit on apple and *A. nemoralis* preferred pear (Sigsgaard, unpublished). Likewise, *A. nemorum* prefer barley to pear for oviposition (Herard & Chen, 1985). Plants may have some dietary value to anthocorids, which might affect oviposition preferences. Thus female longevity of the anthocorid *Orius insidiosus* (Say) -but not fecundity- was higher on young or old leaves of soybean than on middle-aged leaves (Armer *et al.*, 1999). However, oviposition preference of *O. insidiosus* for four plant species was found to correspond with both nymph and adult survival as well as female fecundity, with bean being the optimal plant (Coll, 1996). Both anthocorids have been observed to pierce

leaves (Sigsgaard, personal observation), and so will be exposed to plant substances, which may affect preferences.

If females place eggs close to a food source for the young, this can ensure a better survival of the next anthocorid generation. Thus, a female may choose oviposition site guided by cues of prey such as leaf damage and honeydew as well as the presence of prey itself.

Regarding plant cues, psylla infested pear trees have been shown to be attractive to anthocorids (Scutareanu *et al.*, 1996), and specific volatiles from infested trees identified with will attract *A. nemoralis*, though a laboratory reared culture did not show such a preference before having experienced these volatiles in the presence of food (Drukker *et al.*, 2000). Whether oviposition is also guided by psylla induced plant damage was not tested. However, oviposition preference guided by prey cues have been shown in another predator,

Honeydew may be an attractant as well as a source of water and/or energy for predators (Heidari & Copland, 1993; Patt *et al.*, 2003); (Lakshmi *et al.*, 2000).

The effect of presence of prey will depend on prey dietary quality and prey preferences. Though, both anthocorids are polyphagous predators preying on aphids, mites, psyllids and lepidopteran eggs and young larvae, and are considered to play an important role in controlling insect pests (Hill, 1957; Anderson, 1962a; Anderson, 1962b; Collyer, 1967; Solomon, 1982; Solomon *et al.*, 2000), *A. nemoralis* is considered particularly important in controlling pear psyllids (Fauvel *et al.*, 1984; Solomon *et al.*, 1989; Trapman & Blommers, 1992; Rieux *et al.*, 1994; Scutareanu *et al.*, 1999; Beninato *et al.*, 2000; Solomon *et al.*, 2000). Pear psyllid, *Cacopsylla pyri* L. (Homoptera:

Psyllidae), which is both a preferred and high quality prey to *A. nemoralis* (Dempster, 1963; Fauvel *et al.*, 1984; Hodgson & Aveling, 1988).

The present study tested whether a) *A. nemorum* and *A. nemoralis* would have any oviposition preference with regard to simulated insect damage and whether the oviposition preference of *A. nemoralis* would be affected by b) honeydew and c) the presence of prey, *C. pyri* eggs.

## **Materials and methods**

### *Plant material*

Pear branches (cv. Clara Frijs) were field collected immediately before experiments at Pometet, an experimental orchard belonging to The Royal Veterinary and Agricultural University. Before being introduced to cages branches were gently shaken and individual leaves thereafter carefully examined under a stereomicroscope, while still on the branch. Any remaining arthropods were removed with a fine paintbrush. Finally, branches were washed with demineralized water and air-dried. For experiments, individual healthy and undamaged leaves were selected measuring 3-4 cm in length and 2-3 cm in width.

Oviposition choice was assessed in experimental units with two single leaves. In all cases one leaf was healthy and undamaged. The other leaf was either damaged, had honeydew on it or was infested with *C. pyri* eggs.

### *Insects*

*Anthocorids* *Anthocoris nemorum* females were field collected in and around orchards five to ten days prior to an experiment and up till the onset of the experiment they were kept in thermo cabinets providing L16:D8 with temperatures of  $20\pm 1^{\circ}\text{C}$ . *Anthocoris nemoralis* were laboratory reared in thermo cabinets under the same light and temperature regime. The *A. nemoralis* culture was initiated on individuals obtained from a laboratory rearing facility (EWH BioProduction).

*Rearing and maintenance of anthocorids* For rearing and maintenance anthocorids were kept in units 7 cm in diameter and 8 cm high. The lid was provided with a 2.5 cm in diameter ventilation hole, covered with filter paper. The bottom of units was covered with filter paper. A twice-folded filter paper was provided for hiding. Water was provided on two 2 by 2 cm pieces of moistened paper bandage. Leaves of *Pilea peperomoides* Diel (Urticaceae) were provided for oviposition and additional moisture. To reduce the risk of contamination of cages, leaves were soaked in 2% sodium hypochlorite for 2 min, rinsed with distilled water and allowed to air dry on clean paper prior to use. Biweekly, fresh leaves were provided and old leaves removed. Leaves with eggs were moved to new cages ensuring anthocorids of equal age in individual cages. At the same times excess of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs were provided. Gauze was changed regularly. A maximum of ten adults were kept in each cage.

To ensure that all females had mated, pairs of one male and one female anthocorid were transferred to small units (30 ml 'medicine cups') five to six days prior to oviposition experiments. Units measured 3.5 cm in diameter at the base, 4 cm in

diameter at the top and 4 cm in height, and had lids with ventilation holes. Water was provided on a  $1 \times 1$  cm piece of gauze. In the case of *A. nemoralis* one to two day old females were used. Normally, mating was observed within a few minutes. When females were one week old they were used in the experiment. The females should then be ready to oviposit (Horton *et al.*, 2000).

*Cacopsylla pyri* Newly laid eggs of *C. pyri* were obtained from a greenhouse where *C. pyri* were kept in sleeve cages on potted pear trees. Temperature was set to 20°C but ranged from approximately 17 to 27. Daylength was maintained at L:D 16:8 by supplementing natural daylight with plant lamps.

*Honeydew from C. pyri* Fresh honeydew (up to 24 h old) was collected with a micropipette from honeydew droplets produced by *C. pyri* nymph allowed to feed under a sleeve cage in the greenhouse.

### *Experimental units*

The small units used for mating of anthocorids, as described above, were also used as experimental units. To provide water and support for the leaves the base of the cages were covered with 3% agar, into which the petiole could conveniently be stuck.

### *Method for preference experiments*



*Healthy or damaged leaves* When comparing the two anthocorids preference for healthy or damaged leaves, damage by sap-feeding insects such as psyllids was mimicked by piercing leaves with a fine (minutien) pin. Each leaf had six equidistant pinholes along the midvein (at approx. 2 mm from the midvein) immediately before the experiment. A few females did not oviposit, and could thus not be included in the analysis.

*Honeydew* An experiment assessed oviposition as affected by honeydew. Freshly collected honeydew (0.5 µl) was applied with a micropipette along the ventral midvein of pear leaves leaving four to five small droplets.

*Presence of prey* Finally an experiment was carried out to assess oviposition as affected by the presence of prey eggs. Ten eggs were carefully removed from a leaf and then applied to each treated leaf with a wet paintbrush size 00. All eggs were placed along the ventral midvein. This is similar to the natural distribution of eggs, which are often laid along the midvein by the *C. pyri* female, as well as by the closely related *C. pyricola* Foerster (Horton, 1990). A preliminary experiment with eggs placed like this (n = 5) showed that eggs did not deflate or roll off the leaf. After a week all *C. pyri* eggs had hatched normally.

The dorsal leaf side tends to be the least preferred for oviposition on pear (Sigsgaard, unpublished). To assess, how oviposition would be affected by placing prey eggs on the less-preferred dorsal leaf side, a separate experiment was done. In this case prey eggs were placed along the dorsal midvein of the pear leaf. For this experiment

small leaves were not available, so in order to fit leaves into the small cages each leaf was trimmed from approximately 5 or 6 cm to 4 cm.

At the onset of an experiment one anthocorid female was carefully introduced into each cage. Each experiment lasted 48 h, with L:D of 16:8 and temperature of  $20 \pm 1^\circ\text{C}$ .

After removal of the anthocorids from the cages, plant material was examined for eggs under the stereomicroscope. For each egg, the eggs position was noted as leaf margin ( $< 5$  mm from edge), leaf centre ( $> 5$  mm from edge), leaf tip ( $< 5$  mm from the tip), or midvein ( $< 2$  mm from midvein or on midvein). No eggs were observed in the petiole. It was also noted whether eggs were laid on the dorsal or ventral side of the leaf.

#### *Data analysis*

All statistical analysis was carried out in SAS/STAT ver. 8.2 (SAS Institute, 1990).

Analyses were done using the GLIMMIX-macro with log link and Poisson error.

Glimmix calls PROC MIXED. It allows users to choose an appropriate distribution for the data, and apply a link function. The degrees of freedom of the fixed effects F-tests were adjusted using Satterthwaite formulas. Variance components were estimated by restricted maximum likelihood (REML) (Littell *et al.*, 1996). Anthocorid individuals were included as a random effect. Least significant variables were removed by backward selection starting from full models. Full models included main factors and all possible interaction terms. Approximated t-tests of differences of least square means (LS-means) were used to identify significant differences. Test for covariance components associated with random effects (anthocorid individuals and residuals) showed that anthocorid individuals accounted for a smaller portion (in all cases less than 10%) of random effects.

Replicates in which anthocorids did not lay any eggs during the 48 hours of the assay were excluded from analyses.

## Results

### *Oviposition preference for damaged or healthy leaves*

To test oviposition preference for damaged leaves 25 *A. nemoralis* and 22 *A. nemorum* females were used. Of these seven and five females respectively did not oviposit and were excluded from the analysis leaving 18 replicates with *A. nemoralis* and 17 with *A. nemorum*. None died.

Oviposition preference for damaged leaves was clear for *A. nemoralis* (Figure 1a), while no preference was found for *A. nemorum* (Figure 1b). The crossed effect of treatment  $\times$  anthocorid species was significant ( $F = 5.65$ ,  $df = 1, 65$ ,  $P = 0.021$ ). Differences of LS-means showed that *Anthocoris nemoralis* laid significantly more eggs on damaged leaves than healthy leaves (t-test  $t = 2.97$ ,  $df = 65$ ,  $P = 0.0042$ ), while *A. nemorum* showed no significant preference ( $t = -0.09$ ,  $df = 65$ ,  $P = 0.93$ ).

*Anthocoris nemorum* clearly preferred leaf margins, and *A. nemoralis* leaf blade and vein (Figure 1). A more detailed analysis aiming at investigating if egg distribution on the leaf was affected by leaf damage, showed a significant effect of leaf region  $\times$  anthocorid species ( $F = 4.04$ ,  $df = 1, 374$ ,  $P = 0.045$ ). Differences of LS-means showed that *A. nemoralis* laid significantly more eggs on the ventral leaf blade than on the ventral leaf margin ( $t = 1.97$ ,  $df = 376$ ,  $P = 0.049$ ), and significantly less eggs on the ventral leaf

margin that *A. nemorum* did ( $t = -2.40$ ,  $df = 397$ ,  $P = 0.017$ ). *Anthocoris nemorum* laid significantly more eggs on the ventral leaf margin than on the ventral leaf midvein and leaf blade ( $t = 2.25$ ,  $df = 376$ ,  $P = 0.025$  and  $t = 2.42$ ,  $df = 376$ ,  $P = 0.016$ ) and also laid more eggs on the dorsal leaf margin than on the dorsal midvein and leaf blade ( $t = 2.83$ ,  $df = 376$ ,  $P = 0.005$  and  $t = 3.49$ ,  $df = 376$ ,  $P = 0.0005$ ). Finally, *A. nemorum* laid more eggs on the ventral than on the dorsal leaf margin ( $t = -2.33$ ,  $df = 176$ ,  $P = 0.021$ ). For *A. nemoralis* no significant difference in eggs numbers between leaf sides was found in either leaf margin, leaf blade or midvein. There was no significant effect of anthocorid species  $\times$  treatment  $\times$  leaf location or of treatment  $\times$  leaf region. Thus, the anthocorids choice of leaf region was not significantly affected by treatment.

#### *Oviposition as affected by the presence of honeydew*

The preference for honeydew treated leaves over healthy leaves was tested with 33 *A. nemoralis* females. One died and one did not lay eggs, leaving 31 to be included in the analysis.

*Anthocoris nemoralis* laid more eggs on leaves with honeydew than leaves without honeydew (Figure 2). The preference was highly significant ( $F = 13.54$ ,  $df = 1, 61$ ,  $P = 0.0005$ ). To assess if egg distribution on the leaf was affected by honeydew, a more detailed analysis was done. There was a significant crossed effect of egg position  $\times$  treatment ( $F = 2.62$ ,  $df = 5, 331$ ,  $P = 0.024$ ). The approximated t-test of differences of LS-means revealed that there was no significant difference between the number of eggs laid along the ventral midvein on treated and untreated leaves ( $t = -1.17$ ,  $df = 330$ ,  $P = 0.24$ ). On the other hand, there were highly significantly more eggs on the ventral leaf blade of

the treated than the untreated leaf ( $t = 3.79$ ,  $df = 332$ ,  $P = 0.0002$ ). Further, on treated leaves more eggs were laid on the ventral leaf blade than on the dorsal ( $t = 1.72$ ,  $df = 335$ ,  $P < 0.0001$ ), while there was no difference between the number of eggs on the dorsal and ventral leaf blade on untreated leaves ( $t = 0.70$ ,  $df = 330$ ,  $P = 0.48$ ).

*Oviposition preference as affected by the presence of prey eggs*

The effect of presence of prey eggs on oviposition was first tested with *C. pyri* eggs placed along the ventral midvein. 17 *A. nemoralis* females were used. Of these one died and four did not oviposit, leaving 12 replicates for the analysis of data. In the second experiment *C. pyri* eggs were placed along the dorsal midvein. Here 19 females were used. Of these five did not lay any eggs, leaving 14 replicates. In all experimental units where females had oviposited, all *C. pyri* eggs had been consumed, only leaving empty egg shells.

Oviposition preference was different in the two experiments and the effect of treatment  $\times$  prey position was significant ( $F = 5.03$ ,  $df = 1,19.4$ ,  $P = 0.037$ ). A comparison of LS-means revealed that while *A. nemoralis* laid significantly more eggs on infested leaves in the experiment where *C. pyri* eggs was placed along the ventral midvein ( $t = 2.52$ ,  $df = P = 0.021$ ), there was no significant difference in number of eggs laid on infested and healthy leaves in the experiment where *C. pyri* eggs were placed along the dorsal midvein ( $t = -0.35$ ,  $df = 1,19.4$ ,  $P = 0.73$ ) (Figure 3).

In a more detailed assessment of how proximity to prey may affect oviposition, *A. nemoralis* eggs numbers in four regions of the leaves were compared: dorsal and ventral midvein and dorsal and ventral leaf blade (combining the few eggs laid on the leaf

margins with those on the leaf blades). The effect of prey position  $\times$  treatment  $\times$  egg position was highly significant ( $F = 5.73$ ,  $df = 3, 167$ ,  $P = 0.0009$ ).

In the experiment with *C. pyri* eggs placed along the ventral midvein, *A. nemoralis* eggs were concentrated on the ventral leaf side of the infested leaf, in particular along the ventral midvein, in the immediate proximity of prey eggs (Figure 3a). Significantly more eggs were placed along the ventral midvein than along the dorsal midvein. Further the ventral midvein of the infested leaf held more eggs than the ventral leaf blade ( $t = 2.51$ ,  $df = 167$ ,  $P = 0.0132$  and  $t = 2.65$ ,  $df = 167$ ,  $P = 0.0087$ ). This was not the case on the healthy leaf ( $t = -0.82$ ,  $df = 167$ ,  $P = 0.415$ ). On the untreated leaf no difference was found between the number of eggs laid along the dorsal and ventral midveins ( $t = -0.82$ ,  $df = 167$ ,  $P = 0.42$ ). Further, significantly more eggs were laid along the ventral midvein on the infested than on the healthy leaf ( $t = 2.74$ ,  $df = 167$ ,  $P = 0.0067$ ).

In the experiment where prey eggs had been placed along the dorsal midvein, there was no significant difference between the number of eggs laid along the dorsal and ventral midveins of the infested leaves ( $t = 0.17$ ,  $df = 167$ ,  $P = 0.87$ ), while on the healthy leaf, *A. nemoralis* preferred the ventral to the dorsal midvein ( $t = 2.59$ ,  $df = 167$ ,  $P = 0.011$ ). At the same time, significantly more eggs were found on the dorsal than the ventral leaf blade of the infested leaves ( $t = 3.24$ ,  $df = 167$ ,  $P = 0.0014$ ). In accordance, significantly fewer eggs were found along the ventral midvein on the infested leaf than along the ventral midvein of the healthy leaf ( $t = -2.71$ ,  $df = 167$ ,  $P = 0.0075$ ).

The differences in oviposition pattern on infested leaves depending on prey position are reflected in significant differences in the number of eggs laid along the ventral midveins ( $t = 2.72$ ,  $df = 186$ ,  $P = 0.007$ ) (see also figure 3).

In both experiments, more oviposition was concentrated in leaf regions closest to prey, with resulting differences in egg distribution between healthy and infested leaves and between infested leaves with prey placed along the dorsal or ventral midvein.

## Discussion

### *Oviposition preference for damaged or healthy leaves*

A preference to oviposit on damaged leaves could help anthocorids to locate prey in the field, and was clear for *A. nemoralis*. While attraction to psylla infested leaves and volatiles therefrom have been shown to attract *A. nemoralis* (Drukker *et al.*, 1995; Scutareanu *et al.*, 1997), this study shows that oviposition can also be attracted by mechanical leaf damage in the absence of prey. Prey search of another anthocorid, *Orius tristicolor* (White) (Heteroptera: Anthocoridae), has earlier been found to increase when leaves were artificially damaged with a pin. Thus, bean leaves that prior to experiments had been exposed to plant feeding (thrips or spider mites) or artificial damage, elicited increased searching and resulting higher predation success (VanLaerhoven *et al.*, 2000). That prey cues may affect predator oviposition has been documented for syrphids. The syrphids were observed to adjust their oviposition according to aphid density, even when aphids were removed by the start of the experiment (Bargen *et al.*, 1998).

While the oviposition preference of *A. nemoralis* for damaged leaves was clear, no preference for these leaves was found for *A. nemorum*. In an earlier study it was found that *A. nemorum* laid more eggs on apple or pear leaves, where a part was cut off, than on healthy leaves of the same size, while *A. nemoralis* preferred healthy pear and apple leaves to cut leaves (Sigsgaard, unpublished). Most likely, some of the same plant volatiles are released with the two kinds of leaf damage. However, the anthocorids general search and oviposition pattern means that *A. nemoralis* would be more exposed to pin holes along the midvein, since it has been observed to search the leaf midvein half its time (Brunner & Burts, 1975). In contrast, search for prey by *A. nemorum* is concentrated on leaf margins as found on *Brassica oleracea* var. *gongylodes* (90.4%), tobacco (67.3%) and bean *Phaseolus vulgaris* (65.1%) (Lauenstein, 1980). Therefore, *A. nemorum* would be most exposed to a leaf cut, which it would encounter searching the leaf margin.

In addition the more polyphagous *A. nemorum* might be more likely to place its eggs near leaf damage simulating damage by chewing insects than *A. nemoralis*.

The within-leaf distribution of eggs on damaged and healthy leaves was not significantly different. Eggs of *A. nemorum* were predominantly laid on the leaf margin and eggs of *A. nemoralis* on the leaf blade and along the midvein. Only *A. nemorum* laid more eggs on the ventral leaf side, which in an earlier study tended to be preferred leaf side for both species (Sigsgaard, unpublished), which corresponds to observations of its search pattern with 71% of its time spent on the ventral side of pear leaves (Brunner & Burts, 1975). Data also corresponds to records of the two anthocorids general oviposition



pattern on different plants (Elliott & Way, 1968; Hodgson & Aveling, 1988), and for both species seems to reflect their search pattern (Lauenstein, 1980).

*Oviposition as affected by the presence of honeydew*

On leaves treated with honeydew of *C. pyri* *A. nemoralis* laid more eggs. On leaves with honeydew more eggs were laid on the ventral leaf blade, but fewer eggs were found along the midvein itself, than on the control, perhaps reflecting that though *A. nemoralis* prefers to oviposit close to honeydew, it avoids placing eggs in, or too close to it, perhaps to protect the eggs from being contaminated with the honeydew.

Both *A. nemoralis* and *A. nemorum* have been observed to probe and/or feed honeydew from aphids and psyllids in the field and in the laboratory (Sigsgaard, unpublished). Positive responses to honeydew have been observed in other predators, including Heteropterans and the anthocorid *O. tristicolor*. Honeydew may be an attractant, as shown for *Cyrtorhinus lividipennis* (Reuter) (Heteroptera: Miridae) (Lakshmi *et al.*, 2000). It may also be a source of water and/or energy, as shown for two other predators, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Stephens) (Heidari & Copland, 1993; Patt *et al.*, 2003).

In a Dutch study it was shown that anthocorids were attracted to pear trees attacked by pear psyllids, and in a later study that two compounds; methyl salicylate and (E,E)-alpha-farnesene, both released by pear trees attacked by psyllids, are attractants of *A. nemoralis* (Drukker *et al.*, 1995; Scutareanu *et al.*, 1997). However, none of these compounds were identified in honeydew (Scutareanu *et al.*, 2003), so these compounds

can be disregarded, when considering factors responsible for the attraction to oviposit near honeydew.

*Oviposition preference as affected by the presence of prey eggs*

*Anthocoris nemoralis* preferred to oviposit on leaves with *C. pyri* eggs along the ventral midvein compared to healthy leaves. When prey eggs were placed on the dorsal, less-preferred, leaf side, the effect was not significant. However, a more detailed analysis of egg distribution on the individual leaves showed that eggs were preferentially laid close to where prey eggs had been applied, and that in the case where prey was placed along the dorsal midvein more eggs were laid there than along the ventral midvein –opposite to the untreated leaf where most eggs were found along the ventral midvein. A change in oviposition preference as a result of the position of prey was also documented for *A. nemorum*, which preferred barley to pear for oviposition except when prey, *C. pyri*, was offered on pear leaves (Herard & Chen, 1985).

A study assessing *A. nemorum*'s oviposition preference on apple leaves with or without *Operophtera brumata* L. (Lepidoptera: Geometridae) eggs showed a near-significant preference of *A. nemorum* to oviposit on leaves with prey (Sigsgaard, unpublished). Likewise, *A. nemorum* was observed to prefer to oviposit near spider mite colonies (Steer, 1929).

The fact that oviposition preference was clear even at the small scale used in this series of experiments demonstrates that choice of oviposition site functions even down to position of eggs on the individual leaf.

*Predator oviposition preference and biological control*

The ability of the anthocorids to use prey and prey cues such as leaf damage and honeydew to locate a suitable site for oviposition helps assure the offspring will have prey close at hand. The fact that such preference was observed even in the absence of prey, suggest that prey cues may be used in the field to attract predator oviposition. However, many factors probably interact when predators choose a habitat and a place to oviposit. Thus, an attempt to attract predators to traps with methyl salicylate, a chemical known to attract *A. nemoralis* to psylla infested trees (Scutareanu *et al.*, 1997) showed that anthocorids –in the experiment principally *Orius* sp.- were only attracted in early spring (Molleman *et al.*, 1996).

Though results with honeydew look promising, field experiments attempting to raise predator numbers by application of artificial honeydews have not in all cases been successful with regard to heteroptera and anthocorids. Alfalfa plots treated with artificial honeydew (sucrose and water) held higher numbers of adults of an ichneumonid, adult syrphids, *Geocoris* spp. (Heteroptera: Geocoridae) and *O. tristicolor* but not of spiders or adults of two species of nabids, *Nabis americanoferus* Carayon and *N. alternatus* Parshley (Evans & Swallow, 1993). In another study, potato plots treated with artificial honeydew led to lower densities of *N. alternatus* and of *O. tristicolor* (Ben Saad *et al.*, 1976). The observed avoidance of *A. nemoralis* to lay eggs too near to honeydew should be considered in relation to possible use of honeydew as an attractant of this species.

*Anthocoris nemoralis* is currently under evaluation for inoculative and/or inundative releases against pear psylla in various European countries, so far with variable results, often with initial success followed by dispersal away from the release area

(Fauvel *et al.*, 1994; Beninato *et al.*, 2000). Oviposition preference, presence of prey and/or plant related volatiles, may all affect the attraction to a plant, oviposition and later the retention of adult *A. nemorum* and *A. nemoralis* in orchards and thus the success of biological control.

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**Figure legends**

Figure 1. The average number of eggs laid on damaged (white) and healthy (grey) pear leaves by a) *A. nemoralis* (n = 18) and b) *A. nemorum* (n = 17) according to leaf location. While *A. nemoralis* laid more eggs on damaged leaves, the difference was not significant for *A. nemorum*. Leaf damage did not significantly affect the distribution of eggs within leaf regions for either anthocorid.

Figure 2. The average number of *A. nemoralis* eggs according to their position on the honeydew-treated (white) and untreated (grey) pear leaves (n = 31). More eggs are laid on honeydew-treated leaves. Honeydew was placed along the ventral midvein. Near it, on the ventral leaf blade of the honeydew-treated leaf more eggs are laid than in the same position on the untreated leaf.

Figure 3. Distribution of *A. nemoralis* eggs on leaves with prey (eggs of *C. pyri*) along a) the ventral midvein (white) and its control (grey) (n = 12) and b) the dorsal midvein (white) and its control (grey) (n = 14). In a) more eggs are laid on infested leaves, and in both a) and b) more eggs are laid close to prey eggs –along the ventral midvein in a) and along the dorsal in b).

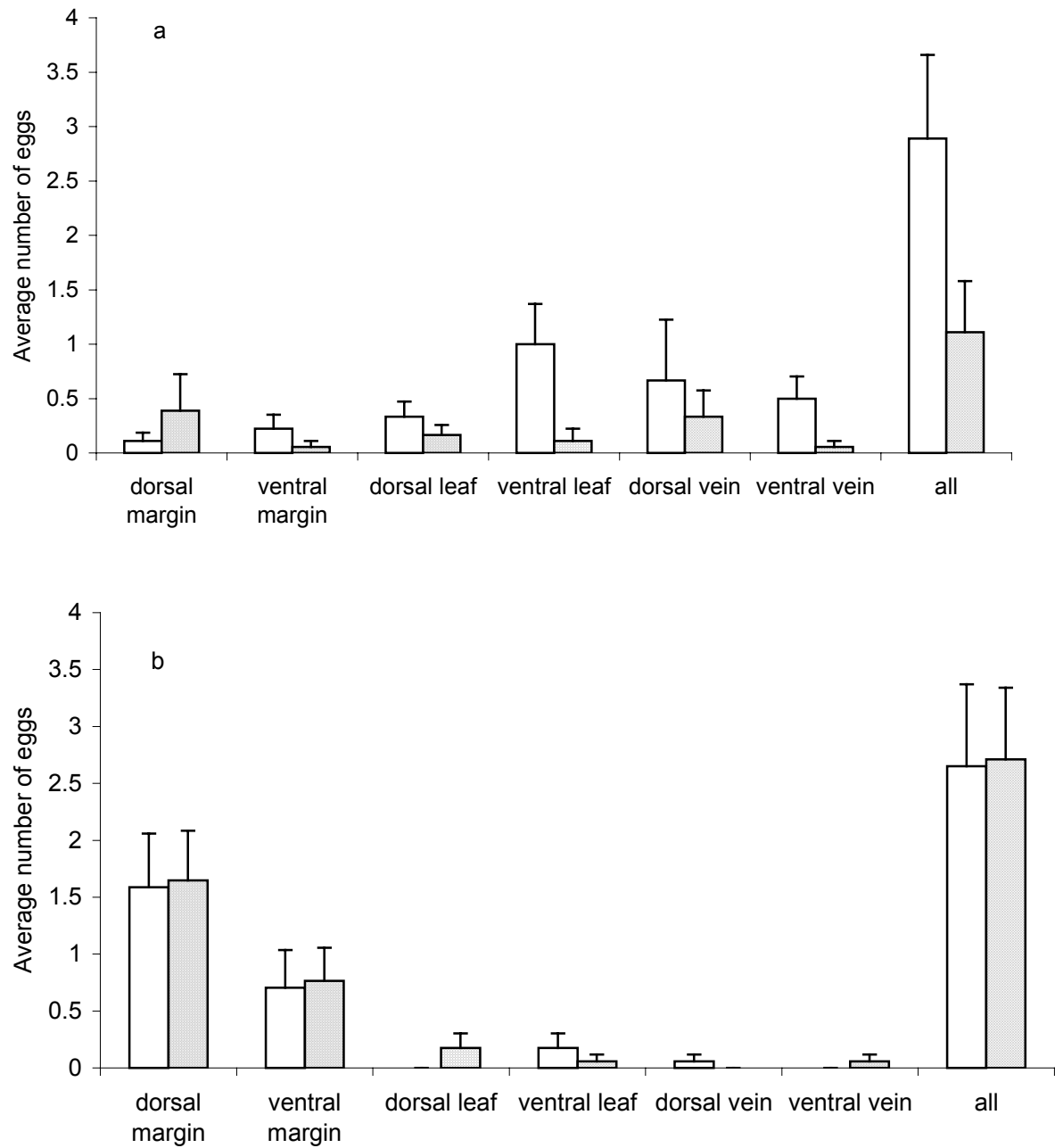
**Figure 1**

Figure 2.

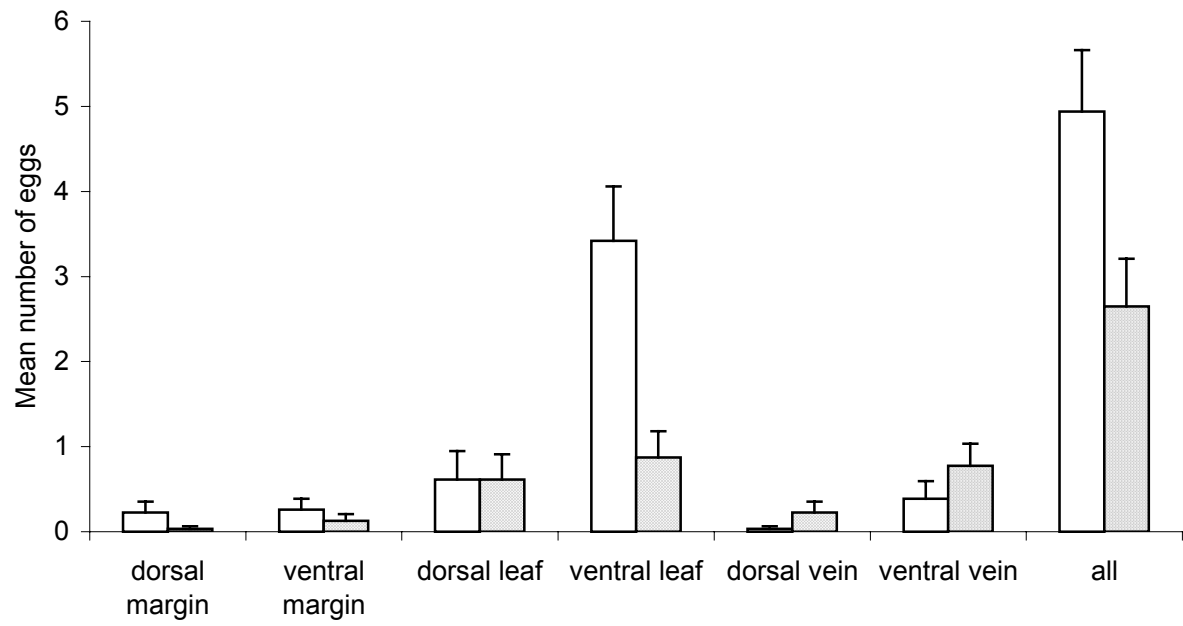


Figure 3

